(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 November 2003 (27.11.2003)

PCT

(10) International Publication Number
WO 03/097927 A1

(51) International Patent Classification[7]: D06M 16/00, C12N 11/08

(21) International Application Number: PCT/PT02/00008

(22) International Filing Date: 21 May 2002 (21.05.2002)

(25) Filing Language: English

(26) Publication Language: English

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/097927 A1

(54) Title: TREATMENT OF ANIMAL HAIR FIBERS WITH MODIFIED PROTEASES

(57) Abstract: A method of treating fibers of animal origin (wool from sheep, cashmere, rabbit, mohair, llama, goat, camel, among others), characterised in that it consists of bringing the fiber into contact with a solution of modified proteases, bonded or not to other substances, in order to increase its molecular weight and reduce its diffusion inside the fiber. It is intended that the cuticle of the fiber be the only accessible part to the protease attack, thus allowing an increase in the resistance to shrinkage and anti-felt finishing, in comparison with untreated material.

DESCRIPTION

"TREATMENT OF ANIMAL HAIR FIBERS WITH MODIFIED PROTEASES"

Field of the invention

The cuticle layer of animal hair fibers presents a scaly structure when observed by microscopy. The felting or shrinkage of these fabrics is due to the overlapping of these scales that surround the cortex (inner part of the fiber), in wet processes with high mechanical agitation. The removal of the cuticle layer makes it possible to eliminate the tendency of the protein fibers of animal origin to shrink. One possibility of anti-felt treatment would be the application of proteolytic treatments for the removal of the cuticle layer. This kind of treatment has been extensively studied since the beginning of the 20th century, but without great achievements.

The reasons for this are primarily due to the following factors:

- Hair fibers of animal origin have a very variable composition, which depends on origin, race, climate and animal feeding. This diversity of animal fibers induces various susceptibilities to proteolytic treatments.

- More aggressive treatments to induce a uniform anti-felting behaviour in all the fibers consequently cause unacceptable loss of strength.

- Recent studies indicate that the lack of reproducibility of the proteolytic treatments and the degradations caused by such treatments are due to the diffusion of the enzymes inside the animal fibers.

Background to the invention

The most commonly used method to confer dimensional stability on articles made from animal hair fibers is the INS/CSIRO Chlorine/Hercosett, which comprises a strong acid chlorine treatment, followed by the application of a polymer resin. This process results in an increased degree of shrinking resistance, but has a number of drawbacks: poor feel, limited durability, difficulties in dyeing and, more importantly today, it generates environmentally damaging waste.

Several authors have suggested methods to reduce the shrinkage of animal fibers, such as wool for instance, which do not result in the release of substances that are harmful to the environment. Among such processes, there are the enzymatic ones, as well as benign chemical processes such as low-temperature plasma treatments. Plasma treatment is a dry process, which involves treating wool fiber material with electric gas discharges (so-called plasma). At present, there are serious obstacles, such as costs, compatibility and capacity, to large-scale commercialisation of a plasma treatment process.

Several enzymatic methods have been used in the treatment of wool. The patent JP-A 51099196 describes a process to treat wool fabrics with alkaline proteases. The patent JP-A 3213574 describes a method for the treatment of wool with transglutaminase or a solution having this enzyme. The patent US 6051033 describes a method of wool or wool fiber treatment with a proteolytic enzyme and tranglutaminase. WO 98/27264 describes a method to reduce the shrinking of wool that consists of bringing the fiber samples into contact with a solution of peroxidase or oxidase under adequate conditions for the enzymatic reaction with wool. The patent US 6099588 relates a method to improve shrink resistance that may result in improvements in feel, appearance

and felting, among others, by the application of proteolytic enzymes in an aqueous solution, after treatment with an alkaline solution containing alcohol. The patent US 5.529.928 refers to a process for obtaining wool with anti-felt finishing, a soft feel and with shrink resistance using an initial chemical oxidation followed by a treatment with protease and warming. The patent EP 134267 uses a similar process, treating the fiber with proteolytic enzymes in the presence of salt, after the initial oxidative treatment. The patent EP 3.58386 describes a method of wool treatment that consists of a proteolytic treatment and one of, or both, an oxidative treatment (such as NaOCl) and treatment with polymer.

The necessity of establishing environmentally friendly (Eco-friendly) methods with better performances than the industrial processes currently used, creates a need for new processes that give a good shrink resistance, softness, appearance and anti-pilling behaviour. Therefore, a new methodology of enzymatic treatment of animal hair fibers is presented here.

Summary of the invention

This invention relates to a new enzymatic process of animal hair fiber treatment, in which the proteases are chemically modified in order to increase their molecular weight and therefore reduce their diffusion inside the fiber. The cuticle will be the only accessible part to the proteolytic attack, which allows for the improvement of one or more wool properties, including their felting and shrinking, without damaging the fiber's interior.

The methodologies used to increase the molecular weight of the enzymes are based on the utilisation of a soluble polymer with hydroxyl groups activated with γ–aminopropyltrietoxysilane and/or glutaraldehyde. The

glutaraldehyde may subsequently bind to another polymer chain, forming a polymeric net, or to an available protein NH_2 group.

Detailed Description and Examples

The method consists of the treatment of the proteic material with a solution of modified proteolytic enzymes. Commercially available proteases from Sigma (*Subtilisin* kind) were used.

Immobilisation was performed on a soluble polymer, polyvinyl alcohol (Sigma), of average molecular weight 70000–100000, using glutaraldehyde (Aldrich), γ–aminopropyltrietoxysilane and/or borax (Sigma) and polyethylenglycol (Sigma) of 10000 of average molecular weight.

The polymer at 6% (w/v) solution in distilled water was dissolved with warming and stirring, activated, and was then added to a 2% (v/v) glutaraldehyde solution. This solution was kept under stirring at room temperature, for 2 hours. After this time, the solution was dialysed in 0.1 M pH 5.0 acetate buffer for 24 hours and then in 0.05 M pH 3.95 acetate buffer for 20 hours.

The enzymatic preparation in the desired concentration was added to the resulting solution, together with PEG (1.25%) and borax (0.05 μg/mL) in 0.1 M pH 5.0 acetate buffer, and kept under stirring for 8 hours at room temperature. This solution was kept at 4°C until use. The immobilisation procedure did not cause any significant loss in activity.

Example 1:

Treatment of pure wool fabric with proteases:

Samples of pure merino wool fabric (like animal hair fiber) of about 12 cm x 12 cm (of about 3 grams each) were placed in a recipient containing a solution of proteases being chemically modified or not, in a relation of 1/20 (w/v). The treatment was performed at 37°C, for periods of time ranging from 4 to 48 hours. The samples were removed from the solution, washed and air-dried. They were then subjected to tests to evaluate possible damage caused during the treatment.

To evaluate the quality of the fabric and the degree of damage caused in the wool treatment process, a qualitative test based on Garner (Garner W., *Textile Laboratory Manual*, vol. 5 - Fibres, 3rd Edition, 1967) was used. It was verified that the modified proteases did not induce fiber degradation when compared with free proteases. The control treatment itself (010 mM pH 7.5 acetate buffer) presents a level of degradation higher than that presented by the fibers treated with modified enzymes.

The tendency of the fabrics to shrink was verified by washing the fabrics (11 x 6 cm) three times in distilled water containing 50 μL of a wetting agent for 60 minutes, at 50°C and 20 rpm, and the shrinkage was measured by the variation of the specimen dimensions. It was verified that only the enzymatically treated fabrics did not induce a significant shrinkage.

A panel of 5 experts evaluated the feel and appearance of the wool fabric and verifyied an increase in the properties of the protease treated fabrics compared to the control fabric.

Example 2:

Treatment of pure wool yarns with proteases:

Similar studies were conducted in yarns of merino wool using the following parameters: samples of pure wool yarn were placed in a recipient containing a solution of proteases being chemically modified or not, in a ratio of 1/20 (w/v). The treatment was conducted at 37°C, for periods of time ranging from 4 to 48 hours. The samples were removed from the solution, washed and air-dried. They were then subjected to tests to evaluate possible damage caused during treatment.

To evaluate the yarn quality and the degree of damage caused in the treatment process of this fiber, a qualitative test based on Garner (Garner W., *Textile Laboratory Manual*, vol. 5 - Fibres, 3rd Edition, 1967) was used. It was verified that the modified protease treatment does not induce degradation when compared with free protease treatment. The control treatment (10 mM pH 7.5 acetate buffer) presented a level of degradation higher than that presented by the fibers treated with the modified enzymes. Tensile strength tests were performed on wool yarns, and it was verified that only the yarns treated with free proteases induced a significant loss of strength.

The tendency to shrink was verified by washing the wool yarns three times in distilled water having 50 μL of a wetting agent for 60 minutes, at 50°C and 20 rpm, and shrinkage was quantified by the visual verification of yarn felting. It was verified that only the enzymatically treated yarns did not induce felting.

A panel of 5 experts evaluated the appearance of the yarns and

verified a better appearance of the yarns treated with proteases, compared to the control yarns.

CLAIMS

1. A method of treating fibers of animal origin (wool from sheep, cashmere, rabbit, mohair, llama, goat, camel, among others), characterised in that it consists of bringing the fiber into contact with a solution of modified proteases, bonded or not to other substances, in order to increase its molecular weight and reduce its diffusion inside the fiber. It is intended that the cuticle of the fiber be the only accessible part to the protease attack, thus allowing an increase in the resistance to shrinkage and anti-felt finishing, in comparison with untreated material.

2. The method according to claim 1, characterised in that it consists of the treatment of the fibers simultaneously with a proteolytic enzyme and transglutaminase or a proteolytic enzyme and glutaraldehyde.

3. The method according to claim 1, characterised in that the proteolytic enzyme is of bacterial origin.

4. The method according to claim 1, characterised in that the proteolytic enzyme is a serine protease.

5. The method according to claims 1 and 4, characterised in that the serine protease is a *Subtilisin Carslberg*.

6. The method according to claim 1, characterised in that the amount of protease used per Kg of wool, fiber or hair is in the range of 1 to 1000 g.

7. The method according to claim 1, characterised in thta the transglutaminase is derived from Streptoverticillium sp.

8. The method according to claim 1, characterised in that a treatment bath with recoverable and reusable protease solution is used, thus reducing the costs of the treatment and the production of effluents, with concomitant savings in water consumption.

9. The method according to claim 1, characterised in that soluble polymers in aqueous solutions, such as polyvinyl alcohol (and/or polymers with hydroxyl groups), are used as supports in the chemical modification of proteases, without restrictions.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/PT 02/00008

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 D06M16/00 C12N11/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 D06M C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 60200 A (NOVO NORDISK BIOCHEM INC) 25 November 1999 (1999-11-25) page 2, line 22 -page 3, line 6 page 8, line 3 - line 25 page 10, line 23 -page 11, line 7 examples 1,6 ---	1-7
X	DATABASE WPI Section Ch, Week 199509 Derwent Publications Ltd., London, GB; Class A35, AN 1995-064089 XP002229518 & JP 06 341067 A (OSAKA PREFECTURE), 13 December 1994 (1994-12-13) abstract --- -/--	1,6,8,9

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
31 January 2003	11/02/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Fiocco, M

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INTERNATIONAL SEARCH REPORT

Internatio ~ pplication No
PCT/PT 02/00008

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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A	US 4 970 156 A (BROUN GEORGES ET AL) 13 November 1990 (1990-11-13) column 2, line 63 -column 3, line 43 column 10, line 13 - line 19 example 9 -----	1-9

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